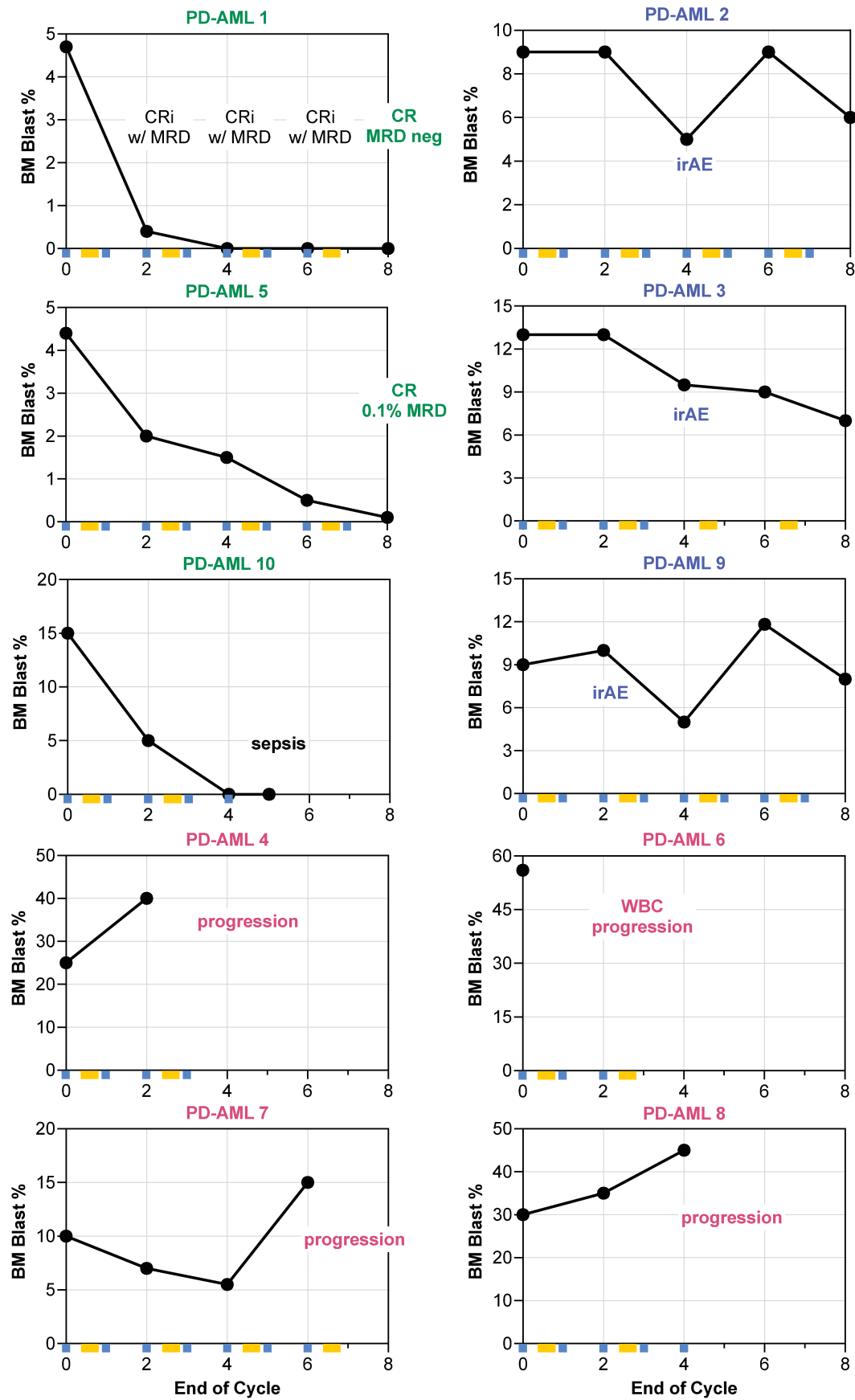
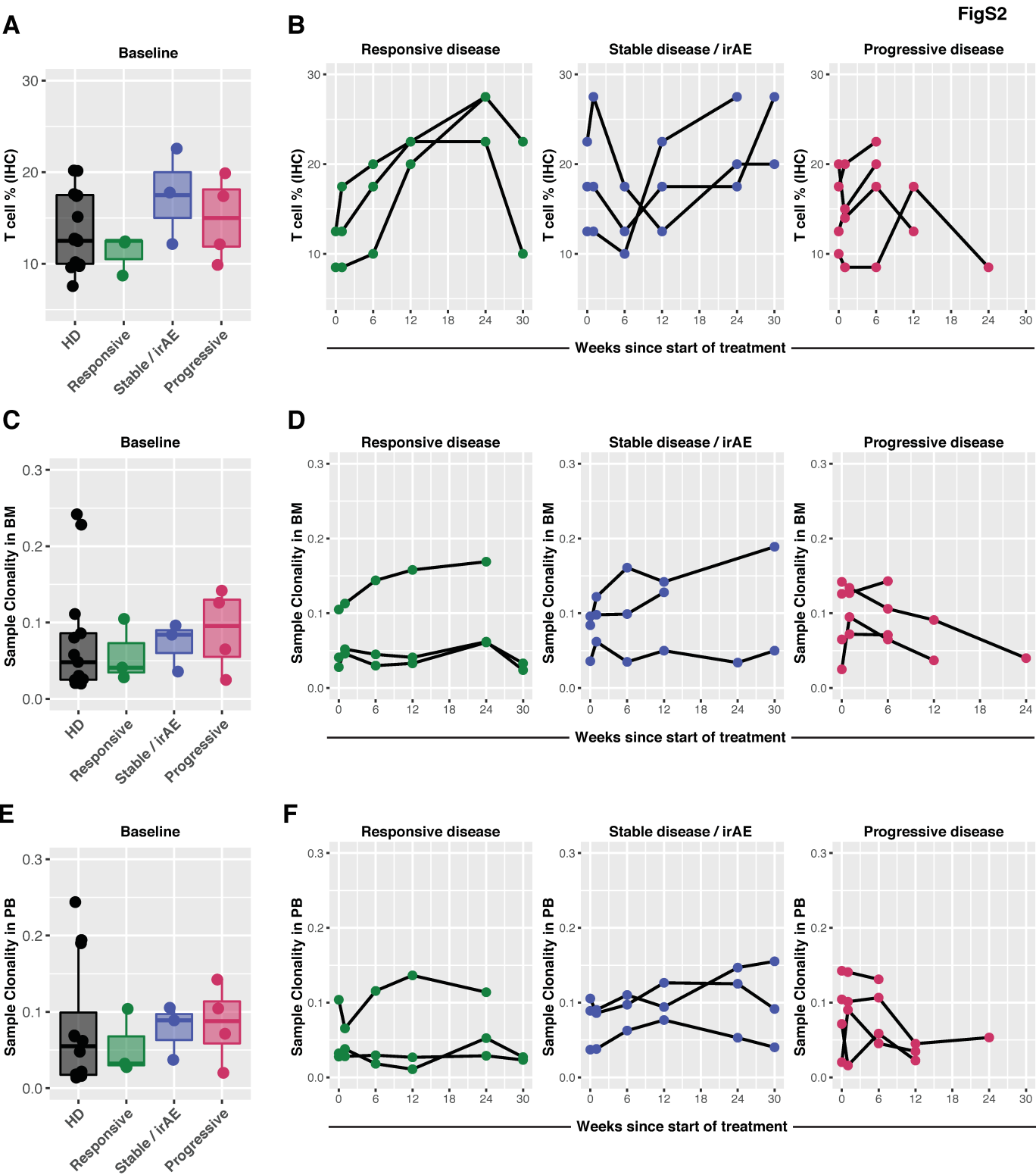


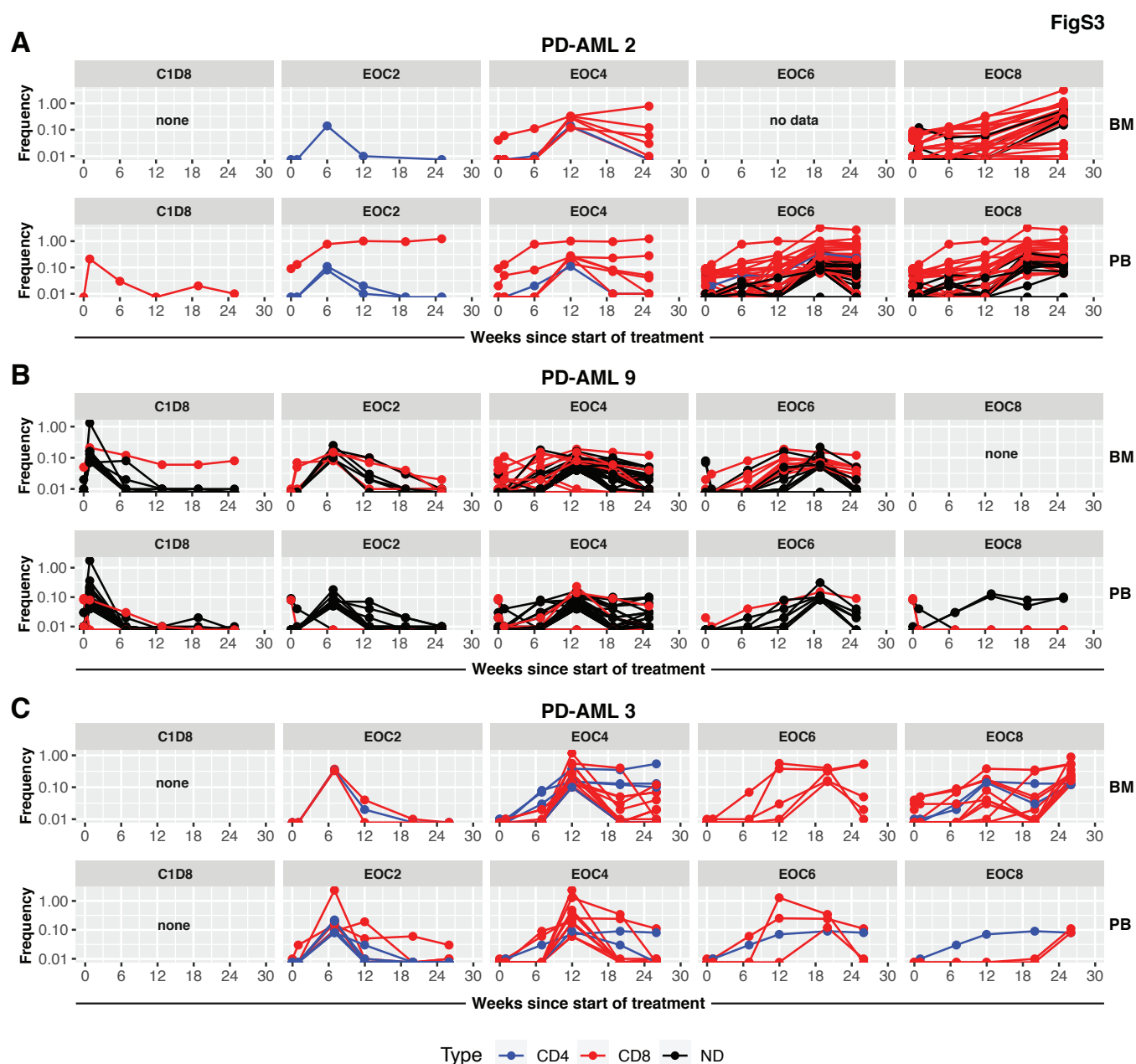
FigS1



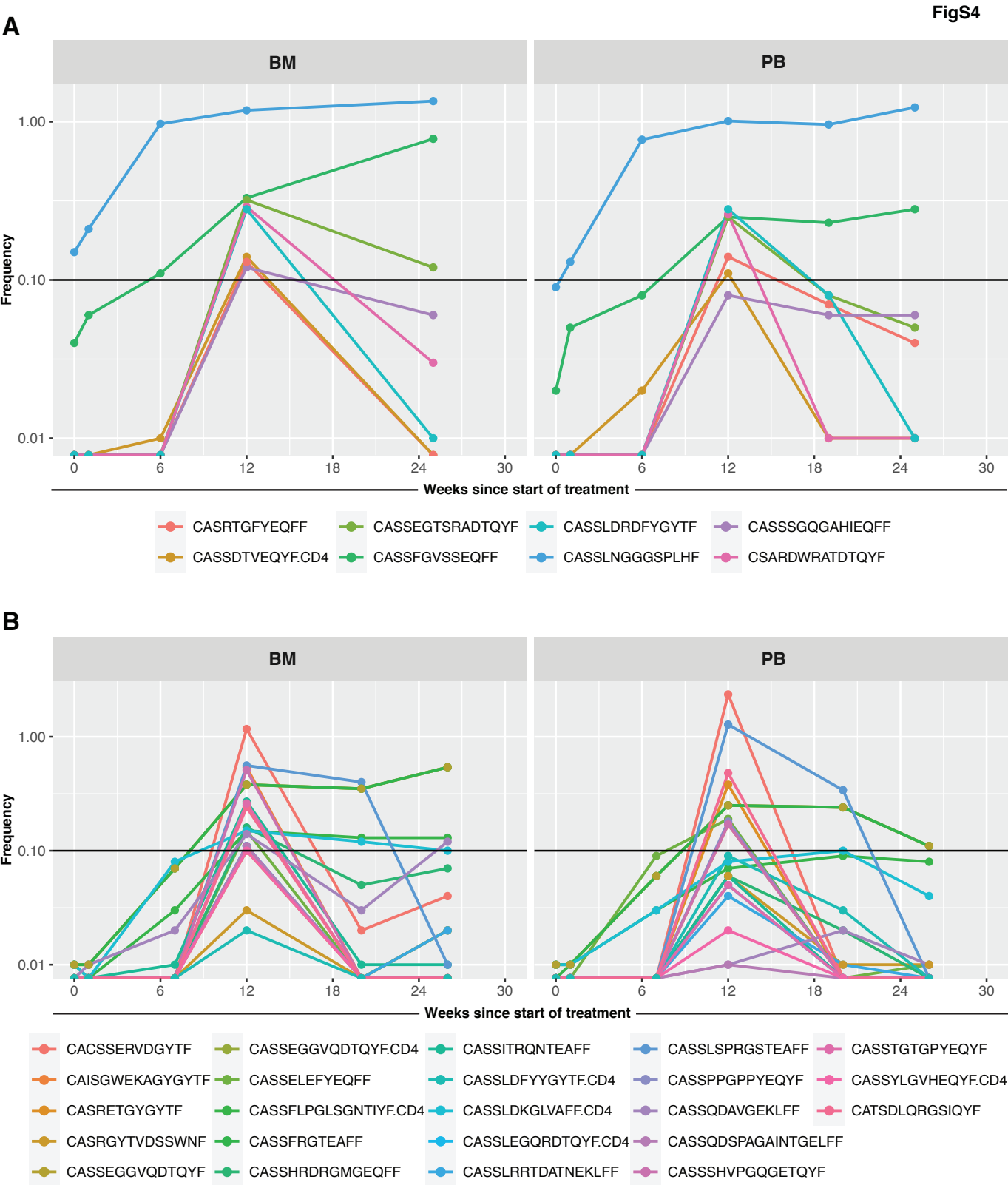
**Fig. S1. Changes in bone marrow leukemic blast burden in patients treated on NCT02996474.** Blast percentages at baseline and after every other cycle of treatment are shown, along with notable clinical events and MRD status, if applicable. Blue squares and along x-axis indicate administered doses of pembrolizumab and yellow rectangles indicate given 10-day dosing of decitabine. Patients with decrease in BM leukemic blast burden (“responsive disease”) are highlighted with green; patients who developed irAEs are indicated in blue, and patients with progressive disease who were taken off study before completion of 8 cycles of treatment are indicated in red.



**Fig. S2. Frequency and diversity of BM-infiltrating and circulating T cells.** (A) Frequencies of CD3+ T cells at baseline in the BM of healthy donors (HD, n=13, previously reported in Williams P, Cancer 2019) and 10 enrolled patients, measured by immuno-histochemistry (IHC) of BM core biopsies. (B) CD3+ T cell frequencies by IHC in BM of patients during treatment. BM TCR $\beta$  reper-toire clonality in HD and PD-AML patients at (C) baseline and (D) during treatment. Circulating PB TCR $\beta$  repertoire clonality in HD and PD-AML patients at (E) baseline and (F) during treatment. For all panels, patients are grouped by response.

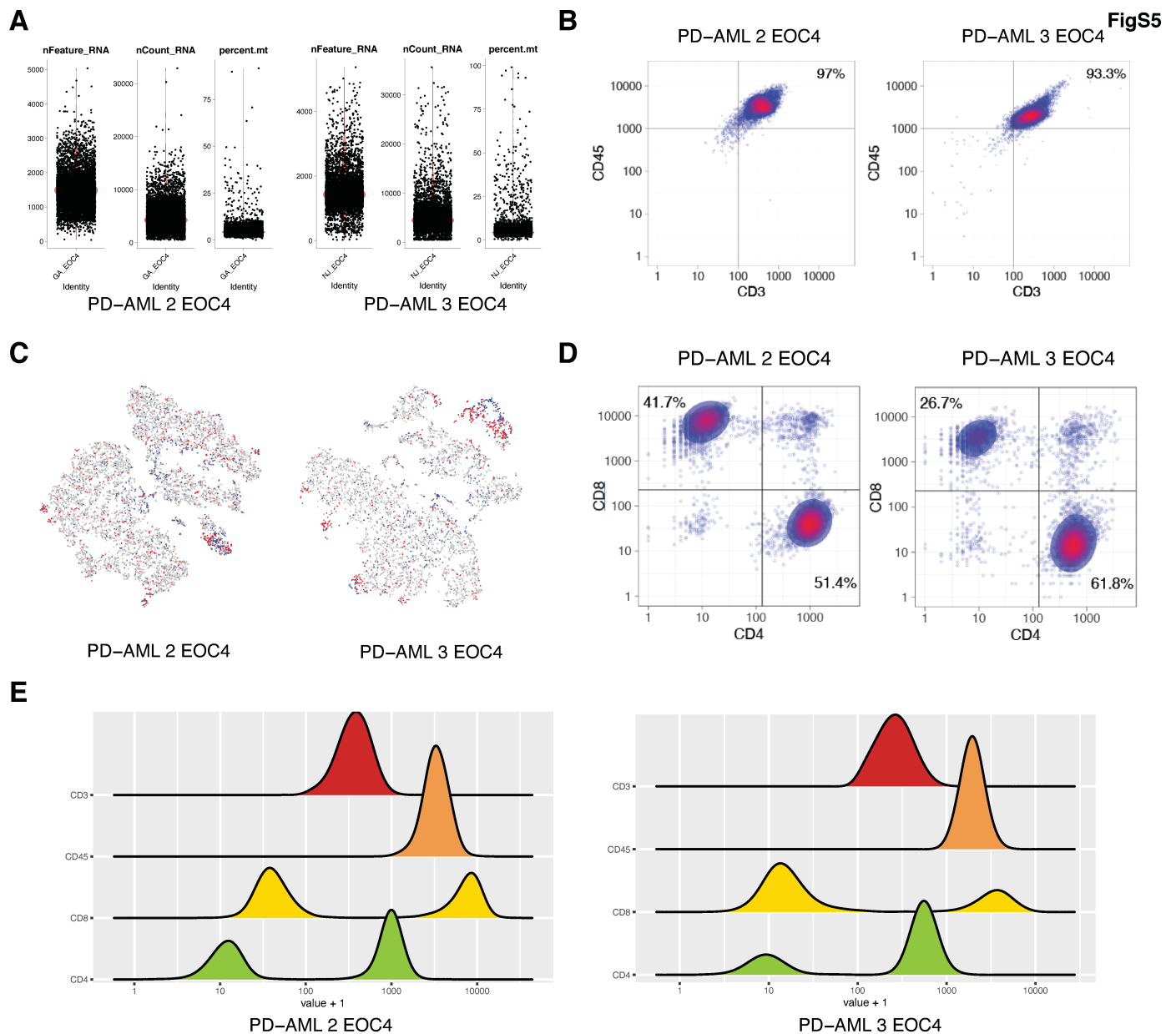


**Fig. S3. Significantly differentially abundant T cell clones in patients developing irAEs.** Pairwise identification of significantly abundant T cell clones between longitudinal timepoints compared to baseline for BM and PB in (A) PD-AML 2 (hypothyroidism at EOC4), (B) PD-AML 9 (hypothyroidism at EOC2), and (C) PD-AML 3 (central diabetes insipidus, at EOC4) was performed. Top and bottom row of each panel shows all significantly abundant clones with a baseline productive frequency of 0.1% or below in BM and PB, respectively. Red indicates CD8+ T cell clones; blue indicates CD8- (CD4+) T cell clones. Black indicates clones where CD4/CD8 could not be determined (ND).

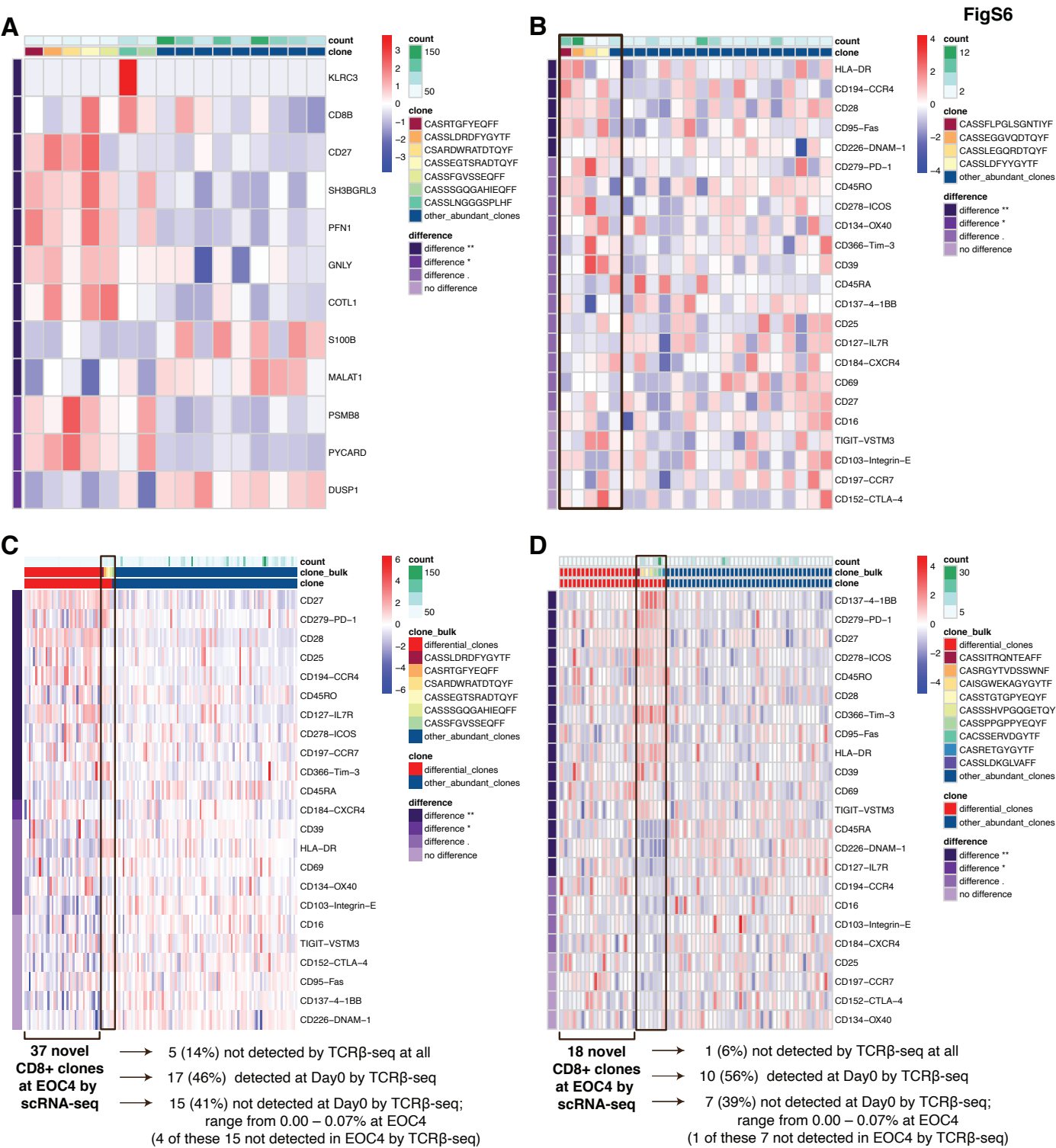


**Fig. S4. Comparable magnitude of expansion of clones of interest in BM and PB in 2 patients developing irAEs.** The frequencies of clones identified as significantly abundant at irAE onset that were low to undetectable in BM and/or PB in (A) PD-AML 2 (B) PD-AML 3. Time since start of treatment in weeks is indicated along x-axis for all plots.

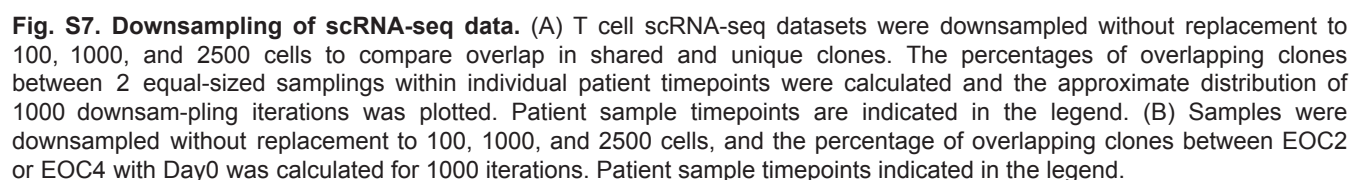




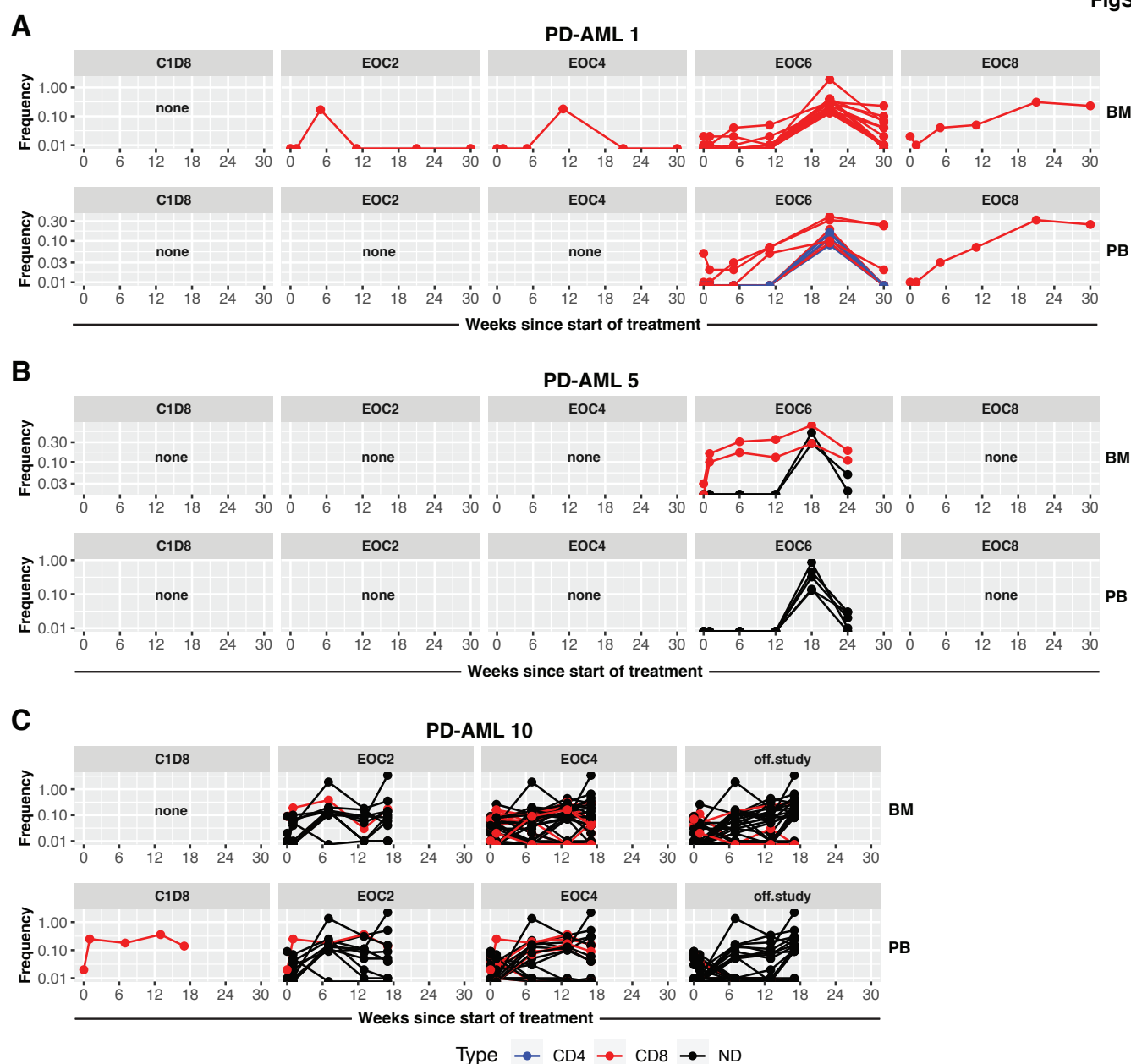
**Fig. S5. Removal of doublets and low-quality cells in 5' scRNA-seq data.** (A) Loose cutoffs for mitochondrial gene percentages and total gene expression level, including number of cell surface feature and total UMI counts, were set in initial filtering. (B) By observing the distribution of CD3 and CD45 expression, a global cutoff was determined for valid T cells (CD3 > 100, CD45 > 1000). (C) To further remove doublets, cells harboring 2 distinct TCR $\beta$  CDR3 sequences (highlighted in red) were removed. Cells in blue indicate cells that had no detected TCR $\beta$  CDR3; these cells were not removed. (D) To remove additional low-quality cells and double negative and double positive cells, the density of CD4 and CD8 expression was plotted and cells below or above both cutoffs (CD4: 130, CD8: 228) were excluded. (E) Cutoffs for all markers were further confirmed by looking at the distributions of markers on ridge plots and by model-based clustering. Data for PD-AML 2 and PD-AML 3 at EOC4 are shown in all panels.



**Fig. S6. Significantly expanded and novel clones detected at EOC4 by scRNA-seq in irAE timepoints.** (A) Differential gene expression profile of significantly expanded clone CASSLNGGGSPLHF in PD-AML 2 EOC4. (B) Cell surface expression profile of significantly expanded CD4+ clones of interest in PD-AML 3 at EOC4. (C-D) Differential cell surface protein expression of novel T cell clones by scRNA-seq in PD-AML 2 at EOC4 and at PD-AML 3 at EOC4, respectively, that were not detected at Day0 or EOC2 timepoints by scRNA-seq. Clones of interest that were identified by bulk TCRβ-seq data of remaining novel clones by scRNA-seq is described. Individual T cell clones, expression scale, cells per clone, and significance level are indicated next to each heat map.

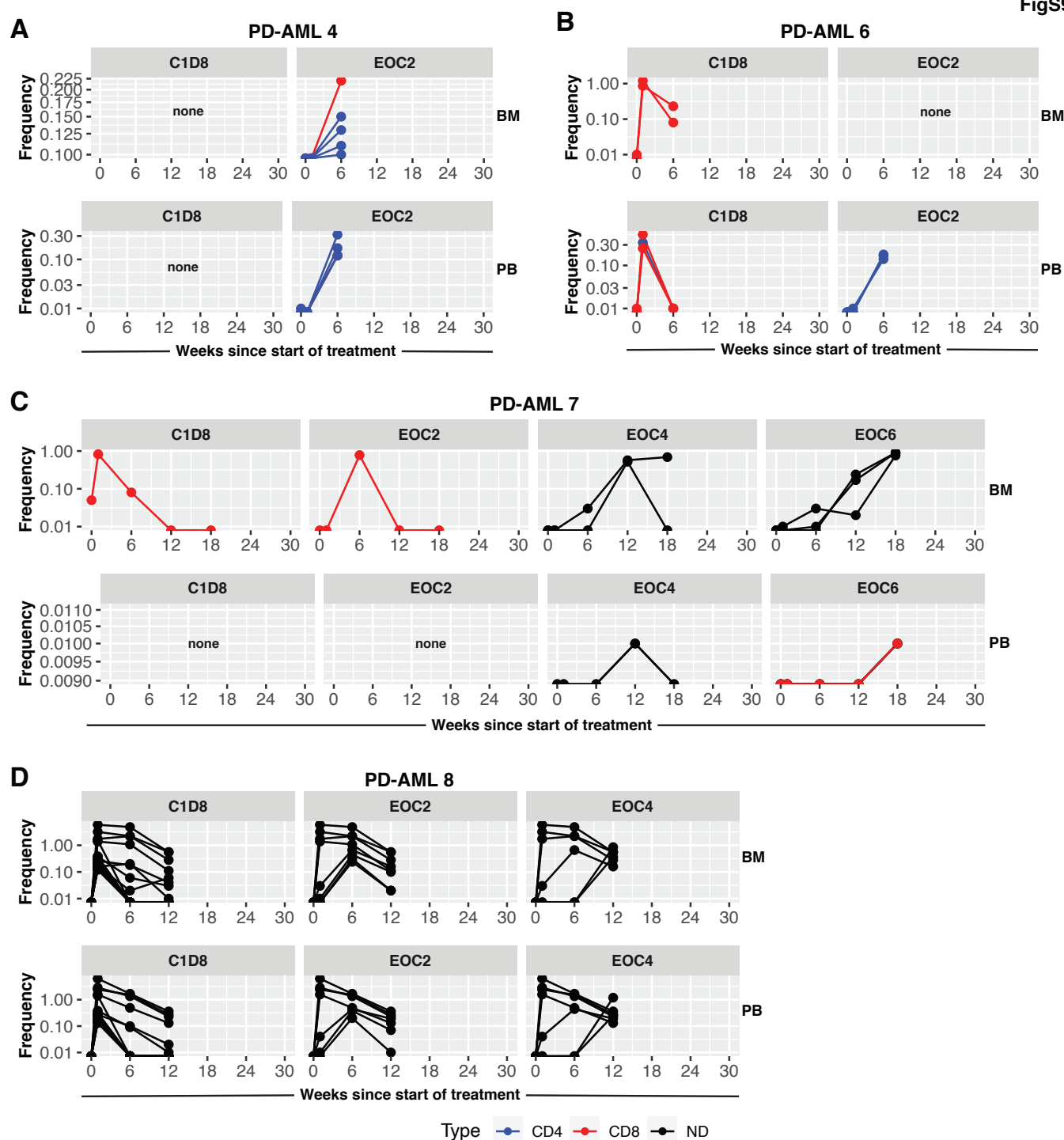


FigS8

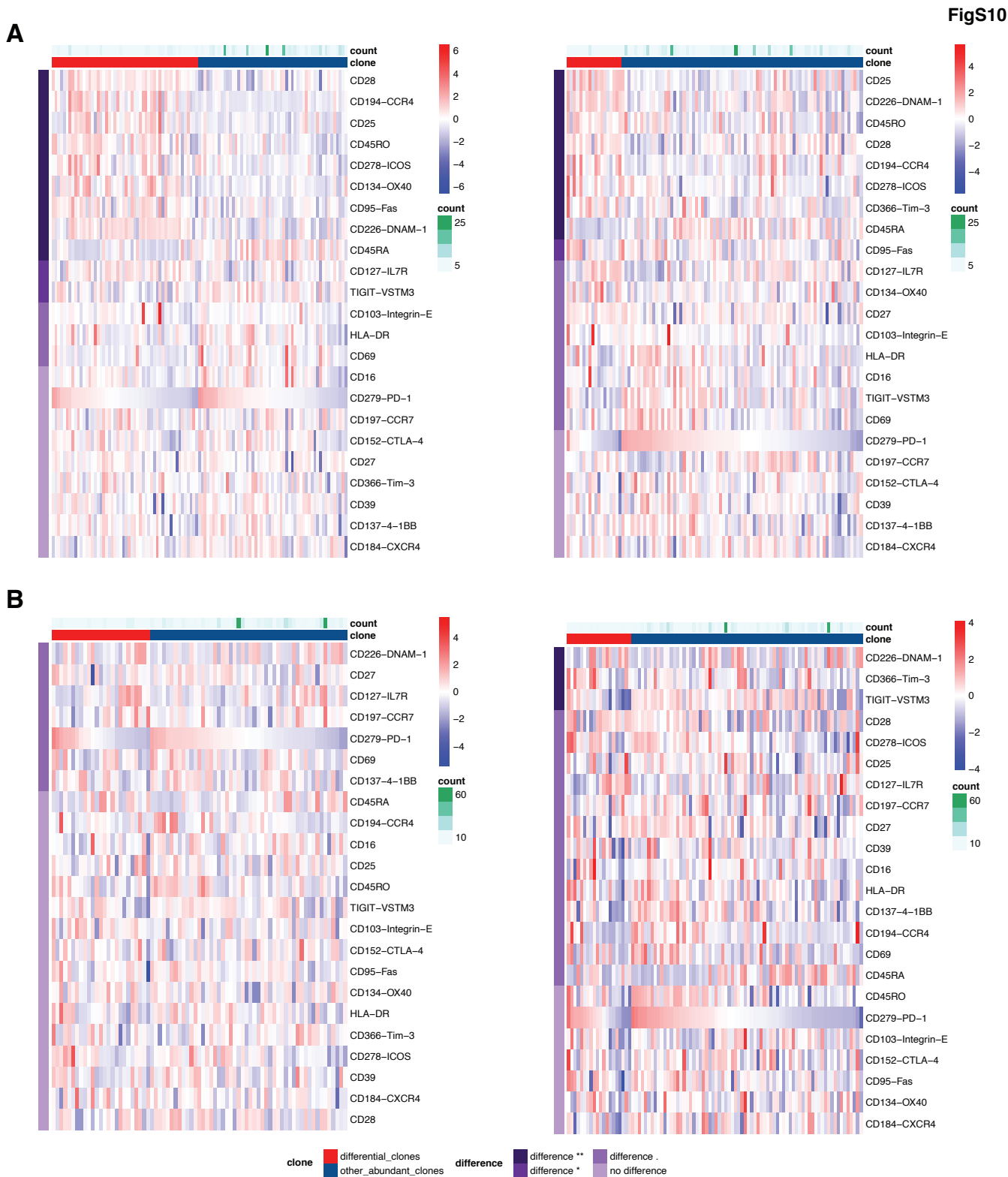


**Fig. S8. Significantly differentially abundant T cell clones in patients with responsive disease.** Pairwise identification of significantly abundant T cell clones between longitudinal timepoints compared to baseline for BM and PB in (A) PD-AML 1, (B) PD-AML 5, and (C) PD-AML 10 (taken off study mid-cycle 5) was performed. Top and bottom row of each panel shows all significantly abundant clones with a baseline productive frequency of 0.1% or below in BM and PB, respectively. Red indicates CD8<sup>+</sup> T cell clones; blue indicates CD8<sup>-</sup> (CD4<sup>+</sup>) T cell clones. Black indicates clones where CD4/CD8 could not be determined (ND).

FigS9

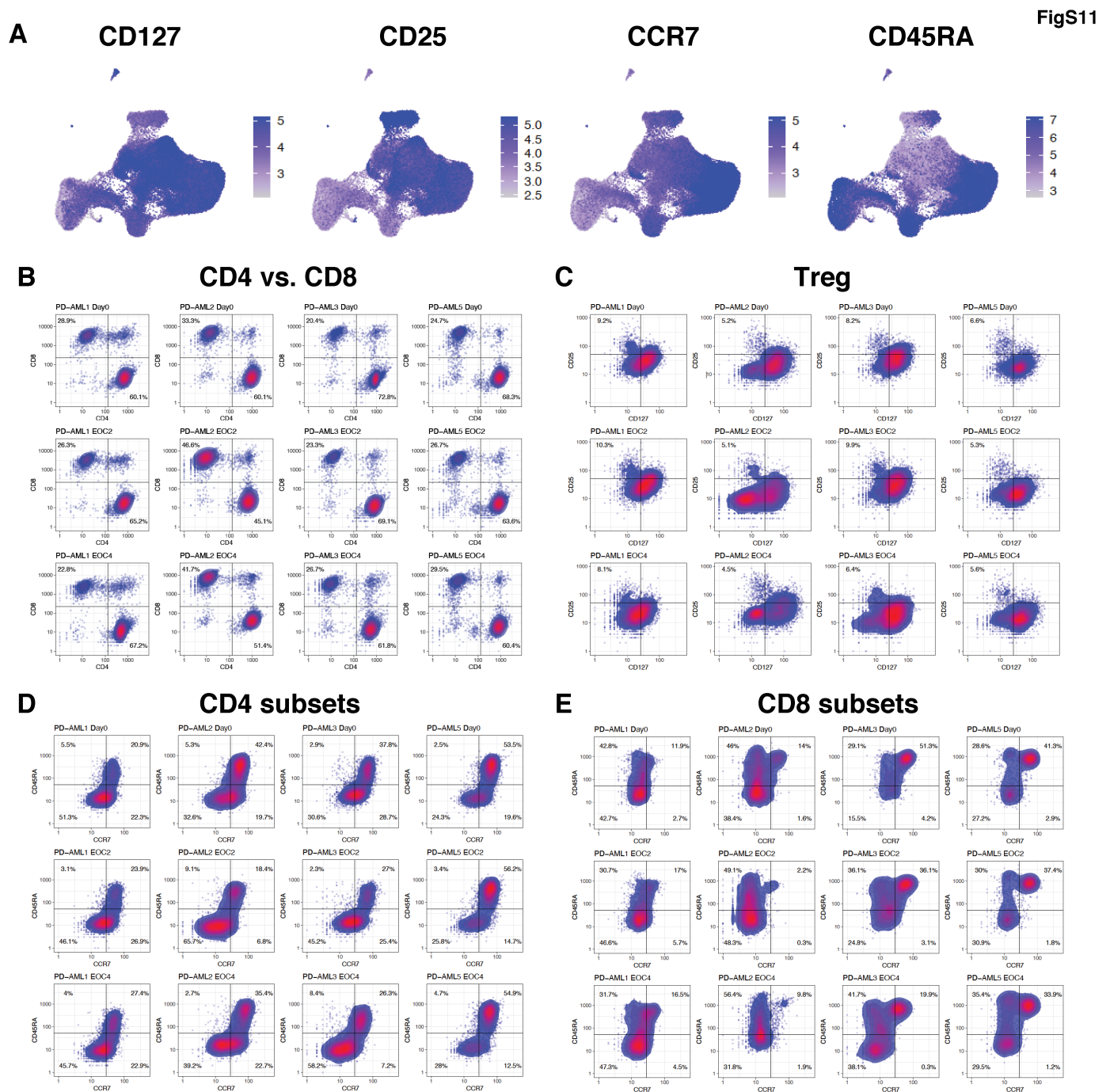


**Fig. S9. Significantly differentially abundant T cell clones in patients with progressive disease.** Pairwise identification of significantly abundant T cell clones between longitudinal timepoints compared to baseline for BM and PB in (A) PD-AML 4, (B) PD-AML 6, (C) PD-AML 7, and (D) PD-AML 8 was performed as described in Fig. S2. Top and bottom row of each panel shows all significantly abundant clones with a baseline productive frequency of 0.1% or below in BM and PB, respectively. Red indicates CD8+ T cell clones; blue indicates CD8- (CD4+) T cell clones. Black indicates clones where CD4/CD8 could not be determined.



**Fig. S10. Putative CD8+ T cell clones discovered by scRNA-seq in responders do not share irAE cell protein signature.** Supervised analysis of DEP between clones detected with scRNA-seq in (A) PD-AML 1 at EOC2 (left) and EOC4 (right) and (B) PD-AML 5 at EOC2 (left) and EOC4 (right) that were not found in the baseline sample of the respective patient. Heatmaps were created using the mean expression of all cells within each clone, and clonotypes are ranked based on relative PD-1 expression. Differentially expressed markers were ordered by adjusted p-values and fold changes. Expression scale and level of difference are indicated by each heat map.

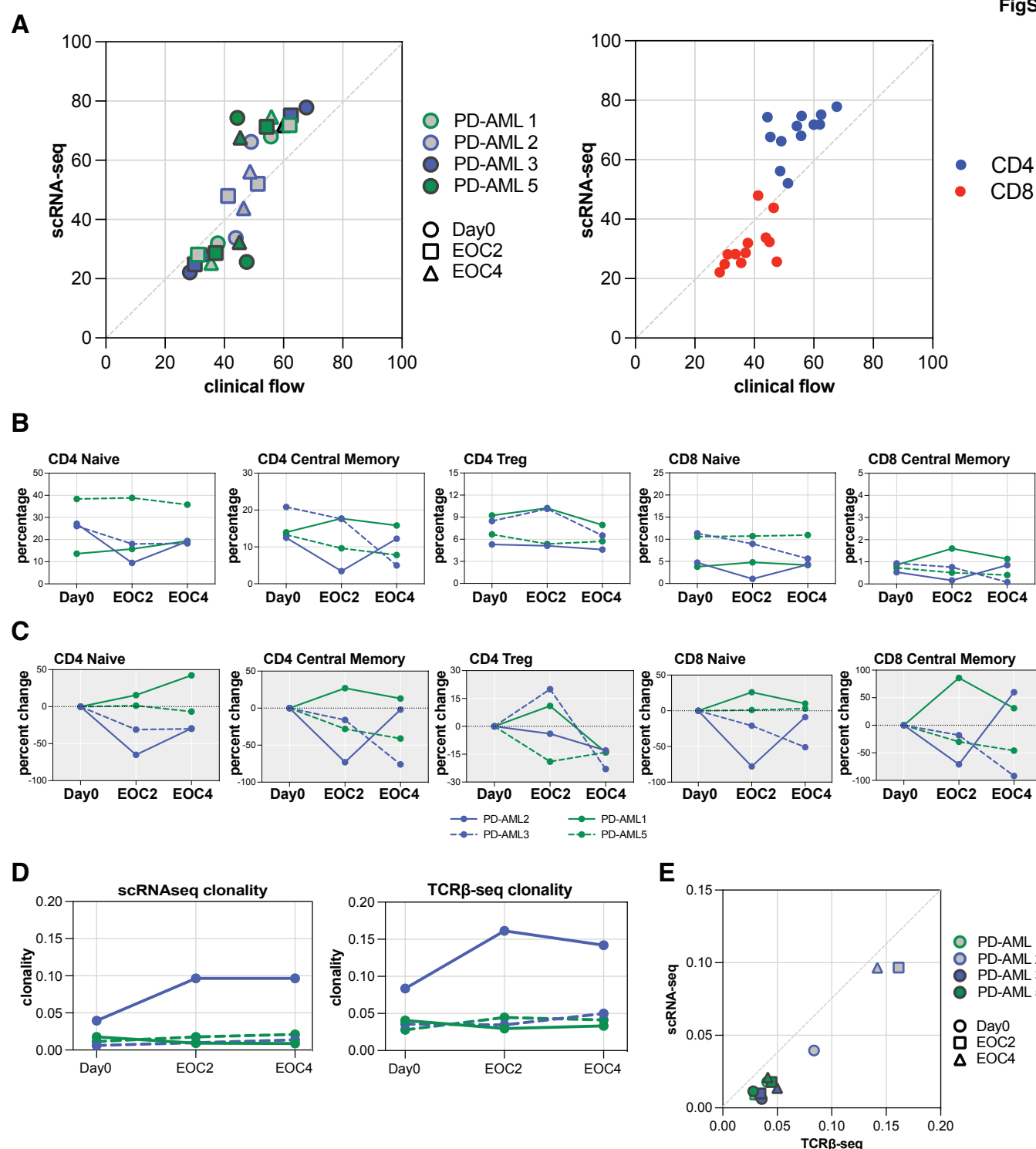




**Fig. S11. Immunophenotyping of 5' scRNA-seq data based on combinatorial cell surface protein expression.** (A) Expression of CD3, CD45, CD4, and CD8 were used in quality control as described in Fig. S4; these markers in addition to CD127, CD25, CCR7, and CD45RA (purple) were used for T cell subset immunophenotyping. Data from all patients and timepoints is visualized. Consensus cutoffs were created by comparing median cutoffs across different timepoints within one sample and adjusting them across all patients. (B) Criteria described in Fig. S4 were applied to remove low-quality, double negative, and double positive T cells based on CD4 and CD8 expression. (C) Tregs were identified based on high CD25 (cutoff >51) and low CD127 (cutoff <26) expression. Naïve (CCR7+CD45RA+), central memory (CM, CCR7+CD45RA-), effector memory (EM, CCR7-CD45RA-), and terminal effector cells (TE) that have re-expressed CD45RA (CCR7-CD45RA+) were gated based on CCR7 (cutoff: 28) and CD45RA (cutoff: 51) expression

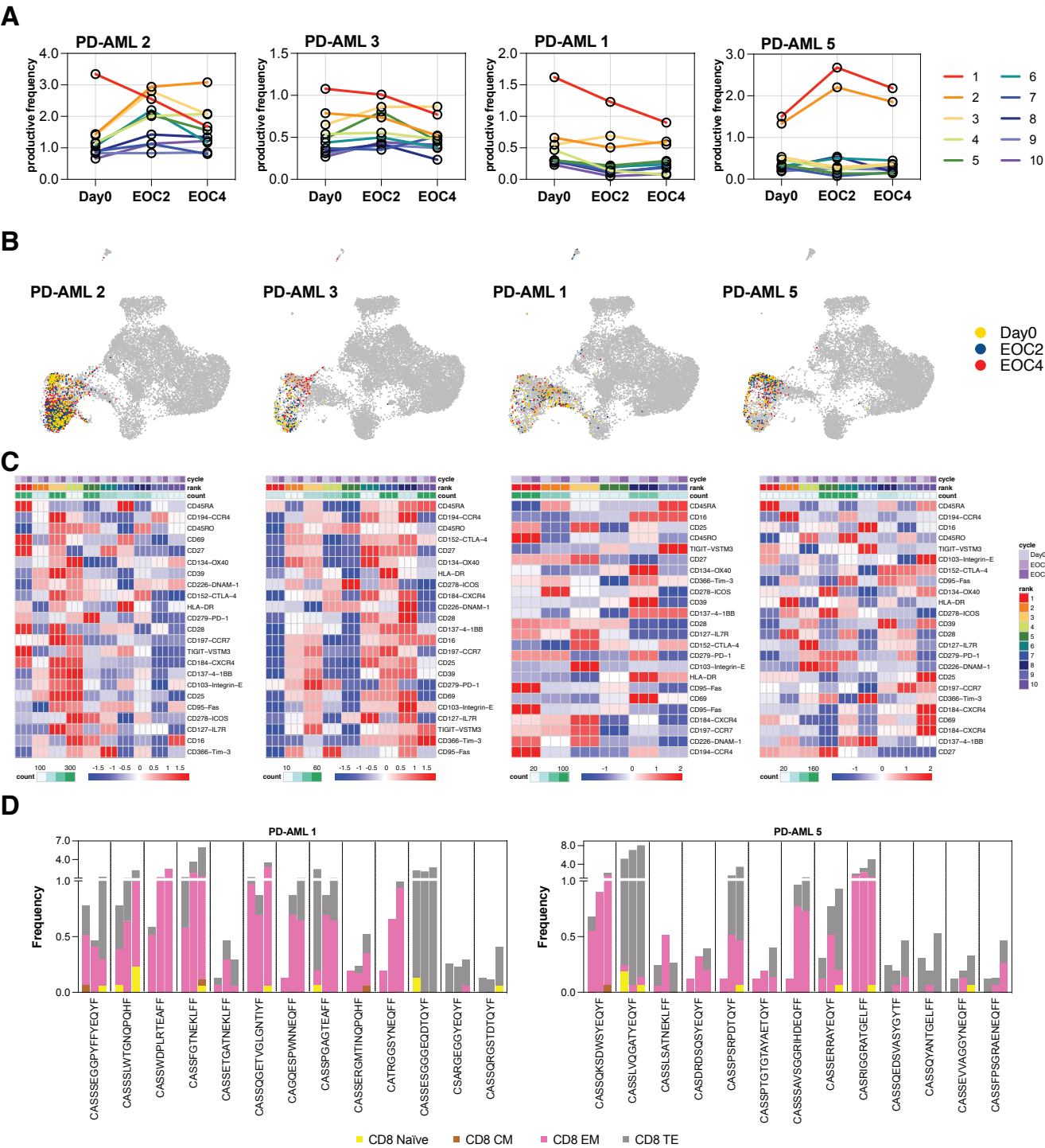


FigS12



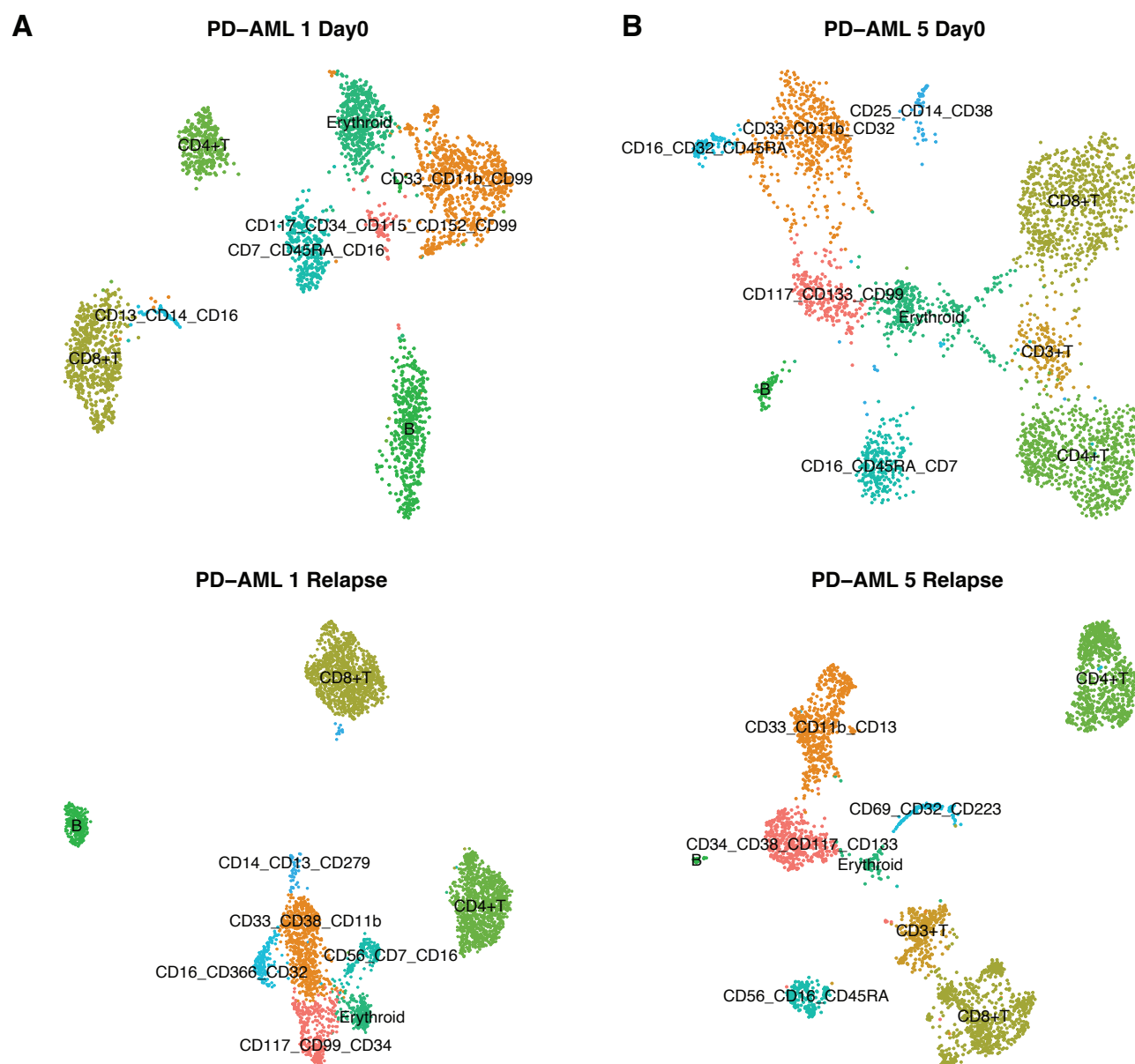
**Fig. S12. Comparison of T cell metrics between scRNA-seq, TCRβ-seq, and clinical flow cytometry.** (A) Correlation of CD4+ and CD8+ T cell frequencies as percent of CD3+ as calculated by scRNA-seq and clinical flow cytometry in patients across timepoints (left), with CD4 or CD8 T cell compartment specified (right). (B) Frequencies of CD4+ and CD8+ naive and central memory (CM) T cells and Tregs at Day0, EOC2, and EOC4, as a percentage of CD3 at each timepoint. (C) Percent change in frequencies of above T cell subpopulations at EOC2 and EOC4 compared to Day0 percentage. (D) Bulk clonality measures calculated from scRNA-seq (left) and TCRβ-seq (right). Patients indicated by legend. (E) Comparison of clonality measures for each sample calculated by both modalities. Patients and timepoints indicated in legend.

FigS13



**Fig. S13. Phenotypes of pre-existing clones during treatment.** (A) Frequencies at Day0, EOC2, and EOC4 of the top 10 most abundant clonotypes at baseline from bulk TCR $\beta$ -seq. Clones are color coded by rank indicated in legend. (B) Visualization of top 10 abundant clones from bulk TCR $\beta$ -seq in scRNA-seq integrated UMAP. (C) Cells containing the same TCR $\beta$  identified from bulk TCR $\beta$ -seq were selected in the scRNA-seq data if the clones were present at all sampled timepoints. Average expression of cell surface proteins from each clone was plotted using normalized count data within each sample. Expression scale, timepoint, and abundant clone rank is indicated by the legend, and the number of detected cells per clone is summarized by the scale below each heat map. (D) Clonotypes were selected from scRNA-seq data from responders if the clonotype was comprised of more than one phenotype (naive, CM, EM, or TE). The frequency of each phenotype per clonotype is plotted with first bar indicating Day0, second bar EOC2, and third bar EOC4. T cell phenotypes indicated in the legend.

FigS14



**Fig. S14. Phenotyping of immune cells and leukemia in 3'v3 scRNA-seq and scDNA-seq.** Clustering based on cell surface protein expression of 3'v3 scRNA-seq acquired from BMBC was performed for (A) PD-AML 1 Day0 (top) and relapse (bottom) and (B) PD-AML 5 Day0 (top) and relapse (bottom). Clusters highly expressing CD3 and CD4/CD8 were labeled as T cells, and those with high CD19 and CD22 expression were annotated as B cells. Erythroid cells were identified as cells with no highly expressed proteins and confirmed by hemoglobin gene expression. For myeloid lineages, clusters with high expression of CD45RA/CD7 and CD16 were considered healthy monocytes. Putative leukemic cells were identified based on expression of known leukemia-associated markers for each patient.

**Table S1. Eligibility assessment and enrollment criteria.**

<b>Inclusion Criteria</b>	• Unequivocal diagnosis of relapsed or refractory acute myeloid leukemia (AML) confirmed by an NIH attending pathologist within 30 days of study enrollment (includes residual AML as confirmed by institutional standards by NIH pathologists).
	• Received at least one prior AML therapy before study enrollment.
	• Ability to comprehend the investigational nature of the study and provide informed consent.
	• Be at least 18 years of age on day of signing informed consent.
	• Availability of a physician willing to assume clinical care after completion of the study.
	• Be willing to provide blood and bone marrow for research as described in the study.
	• Have a performance status of less than or equal to 2 on the ECOG Performance Scale.
	• Demonstrate adequate organ function; all screening labs should be performed within 14 days of treatment initiation.
	• Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
	• Female subjects of childbearing potential must be willing to use an adequate method of contraception for the course of the study through 120 days after the last dose of study medication. Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
<b>Exclusion Criteria</b>	• Male subjects of childbearing potential must agree to use an adequate method of contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy. Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
	• Has a diagnosis of acute promyelocytic leukemia (APL)
	• Has previously received an allogeneic hematopoietic stem cell transplant.
	• Has received AML treatment with an investigational therapy or device within 4 weeks of the first dose of treatment.
	• Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
	• Has a known history of active TB (Bacillus Tuberculosis)
	• Has hypersensitivity to pembrolizumab or any of its excipients.
	• Has hypersensitivity to decitabine or any of its excipients.
	• Has received more than two prior cycles of decitabine.
	• Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1.
	• Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1. Subjects who have received cytoreductive therapy with hydroxyurea at any time prior to study Day 1 are an exception to this criterion.
	• Has not recovered (i.e., $\leq$ Grade 1 or at baseline) from adverse events due to previously administered AML therapy agents. Subjects with $\leq$ Grade 2 neuropathy are an exception and may qualify for the study. If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
	• Has a known additional malignancy that is progressing or requires active treatment e.g.: basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
	• Has known malignant central nervous system (CNS) involvement.
	• Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
	• Has history of (non-infectious) pneumonitis that required steroids, evidence of interstitial lung disease or active, non-infectious pneumonitis.
	• Has an uncontrolled active infection.
	• Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
	• Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
	• Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
	• Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
	• Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
	• Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
	• Has received a live vaccine within 30 days of planned start of study therapy. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

**Table S2. Response and discontinuation criteria.**

Toxicity	Hold for Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea / colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)*	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism		Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2*	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug- Related Toxicity**	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue

\* If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. \*\* Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

**Table S3. Total Seq-C antibodies used with 5' scRNA-seq.**

Specificity	Clone	Barcode Sequence
CD3	UCHT1	CTCATTGTAACTCCT
CD8	SK1	GCGCAACTTGATGAT
CD45RA	HI100	TCAATCCTTCCGCTT
CD194 (CCR4)	L291H4	AGCTTACCTGCACGA
CD4	RPA-T4	TGTTCCCGCTCAACT
CD16	3G8	AAGTTCACTCTTTGC
CD25	BC96	TTTGTCTGTACGCC
CD45RO	UCHL1	CTCCGAATCATGTTG
CD279 (PD-1)	EH12.2H7	ACAGCGCCGTATTTA
TIGIT (VSTM3)	A15153G	TTGCTTACCGCCAGA
CD69	FN50	GTCTCTTGCTTAAA
CD197 (CCR7)	G043H7	AGTTCAGTCAACCGA
CD152 (CTLA-4)	BNI3	ATGGTTCACGTAATC
CD27	O323	GCACTCCTGCATGTA
CD95 (Fas)	DX2	CCAGCTCATTAGAGC
CD134 (OX40)	Ber-ACT35 (ACT35	AACCCACCGTTGTTA
HLA-DR	L243	AATAGCGAGCAAGTA
CD366 (Tim-3)	F38-2E2	TGTCCTACCCAACTT
CD278 (ICOS)	C398.4A	CGCGCACCCATTAAA
CD39	A1	TTACCTGGTATCCGT
CD137 (4-1BB)	4B4-1	CAGTAAGTTCGGGAC
CD184 (CXCR4)	12G5	TCAGGTCCTTTCAAC
CD226 (DNAM-1)	11A8	TCTCAGTGTTTGTGG
CD28	CD28.2	TGAGAACGACCCTAA
CD45	HI30	TGCAATTACCCGGAT
CD103 (Integrin $\alpha$ E)	Ber-ACT8	GACCTCATTGTGAAT
CD127 (IL-7R $\alpha$ )	A019D5	GTGTGTTGTCCTATG

All antibodies from Biolegend.

**Table S4. TotalSeq-A antibodies used with 3'v3 scRNA-seq.**

Specificity	Clone	Barcode Sequence
CD115 (CSF-1R)	9-4D2-1E4	AATCACGGTCCTTGT
CD117 (c-kit)	104D2	AGACTAATAGCTGAC
CD11b	ICRF44	GACAAGTGATCTGCA
CD123	6H6	CTTCACTCTGTCAGG
CD127 (IL-7R $\alpha$ )	A019D5	GTGTGTTGTCCTATG
CD13	WM15	TTTCAACGCCCTTTC
CD133	S16016B	GTAAGACGCCTATGC
CD14	63D3	CAATCAGACCTATGA
CD152 (CTLA-4)	BNI3	ATGGTTCACGTAATC
CD16	3G8	AAGTTCACCTTTTGC
CD184 (CXCR4)	12G5	TCAGGTCCTTTCAAC
CD19	HIB19	CTGGGCAATTACTCG
CD194 (CCR4)	L291H4	AGCTTACCTGCACGA
CD197 (CCR7)	G043H7	AGTTCAGTCAACCGA
CD2	TS1/8	TACGATTTGTCAGGG
CD22	S-HCL-1	GGGTTGTTGTCTTTG
CD223 (LAG-3)	11C3C65	CATTTGTCTGCCGGT
CD226 (DNAM-1)	11A8	TCTCAGTGTTTGTGG
CD244 (2B4)	C1.7	TCGCTTGATGGTAG
CD25	BC96	TTTGTCTGTACGCC
CD26	BA5b	GGTGGCTAGATAATG
CD274 (B7-H1, PD-L1)	29E.2A3	GTTGTCCGACAATAC
CD279 (PD-1)	EH12.2H7	ACAGCGCCGTATTTA
CD3	UCHT1	CTCATTGTAACCTCT
CD32	FUN-2	GCTTCCGAATTACCG
CD33	P67.6	TAATCAGGGCCTAT
CD34	581	GCAGAAATCTCCCTT
CD366 (Tim-3)	F38-2E2	TGTCCTACCCAACTT
CD38	HIT2	TGTACCCGCTTGTGA
CD4	RPA-T4	TGTTCCCGCTCAACT
CD44	BJ18	AATCCTTCCGAATGT
CD45	HI30	TGCAATTACCCGGAT
CD45RA	HI100	TCAATCCTTCCGCTT
CD45RO	UCHL1	CTCCGAATCATGTTG
CD56 (NCAM)	5.1H11	TCCTTTCCTGATAGG
CD69	FN50	GTCTCTGGCTTAAA
CD7	CD7-6B7	TGGATTCCCGGACTT
CD8	SK1	GCGCAACTTGATGAT
CD90 (Thy1)	5E10	GCATTGTACGATTCA
CD95 (Fas)	DX2	CCAGCTCATTAGAGC
CD96 (TACTILE)	NK92.39	TGGCCTATAAATGGT
CD99	3B2/TA8	ACCCGTCCCTAAGAA
CLEC12a	50C1	CATTAGAGTCTGCCA
HLA-A,B,C	W6/32	TATGCGAGGCTTATC
HLA-DR	L243	AATAGCGAGCAAGTA
HLA-DR,DP,DQ	Tu39	AGCTACGAGCAGTAG

All antibodies from Biolegend.



**Table S5. Oligos used in ADT cDNA and library amplification.**

ADT additive primer	5'CCTTGGCACCCGAGAATTCC
SI-PCR primer	5'AATGATACGGCGACCAACGAGATCTACACTCTTCCCTACACGACGCTC
Illumina small RNA primer RPI-2	5'CAAGCAGAAGACGGCATAACGAGATACATCGGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Illumina small RNA primer RPI-3	5'CAAGCAGAAGACGGCATAACGAGATGCCTAAGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Illumina small RNA primer RPI-5	5'CAAGCAGAAGACGGCATAACGAGATCACTGTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA
Illumina small RNA primer RPI-7	5'CAAGCAGAAGACGGCATAACGAGATGATCTGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA

**Table S6. CD19+ B cell frequencies by IHC during treatment.**

Patient	Day0	C1D8	EOC2	EOC4	EOC6	EOC8
PD-AML 1	5	5	3	3	2	2
PD-AML 2	< 1	< 1	< 1	< 1	-	-
PD-AML 3	< 1	< 1	< 1	< 1	< 1	< 1
PD-AML 4	< 1	1.5	< 1	-	-	-
PD-AML 5	1.5	2.5	1.5	1.5	< 1	< 1
PD-AML 6	2	1	1	-	-	-
PD-AML 7	3	2	1.5	1.5	1	-
PD-AML 8	4	4	5	4	-	-
PD-AML 9	< 1	< 1	< 1	< 1	< 1	< 1
PD-AML 10	< 1	< 1	< 1	< 1	< 1 *	-

- denotes unavailable data; \* indicates mid-cycle off-study timepoint for this patient

**Table S7. Anti-thyroid autoantibody levels in patients developing hypothyroidism.**

Patient	Thyroid hormone levels at irAE onset		Baseline antibodies (IU/mL)		Antibodies at irAE onset (IU/mL)	
	TSH	Free T4	anti-TG	anti-TPO	anti-TG	anti-TPO
PD-AML 2	67	0.4	<20.0	<10.0	21.6	19.8
PD-AML 9	22	-	-	-	<20.0	<10.0
Reference Range	0.27-4.2 mIU/mL	0.9-1.7 ng/dL	0.0-40.0 IU/mL	0.0-34.9 IU/mL	0.0-40.0 IU/mL	0.0-34.9 IU/mL

- denotes unavailable data; values in red outside normal ranges; TSH, thyroid stimulationg hormone; T4, thyroxin; TG, thyroglobulin; TPO, thyroid peroxidase

**Table S8. QC of 5'scRNA-seq data with removal of cells with low CD3/CD45, and both low or both high CD4/CD8 expression.**

Patient	Timepoint	Acquired Cell Number	RNA count*	>1 CDR3**	CD3/CD45 †	CD4/CD8 ‡	Final Cell Number
PD-AML 2	Day0	5480	258	437	263	184	4338
	EOC2	6011	264	574	450	266	4457
	EOC4	8612	457	735	241	363	6816
PD-AML 3	Day0	7193	286	612	154	239	5902
	EOC2	5245	81	435	153	235	4341
	EOC4	5519	644	466	302	225	3882
PD-AML 1	Day0	6084	363	460	258	365	4638
	EOC2	6548	275	356	391	372	5154
	EOC4	6842	426	547	370	358	5141
PD-AML 5	Day0	6071	310	355	308	205	4893
	EOC2	6006	285	437	442	182	4660
	EOC4	5695	239	395	309	231	4521

\* removal based on mitochondrial gene content and UMI counts; \*\* removal of cells containing more than 1 TRB gene; † removal of cells without cell surface expression of CD3 and CD45; ‡ removal of cells expressing both or no cell surface CD4 and CD8